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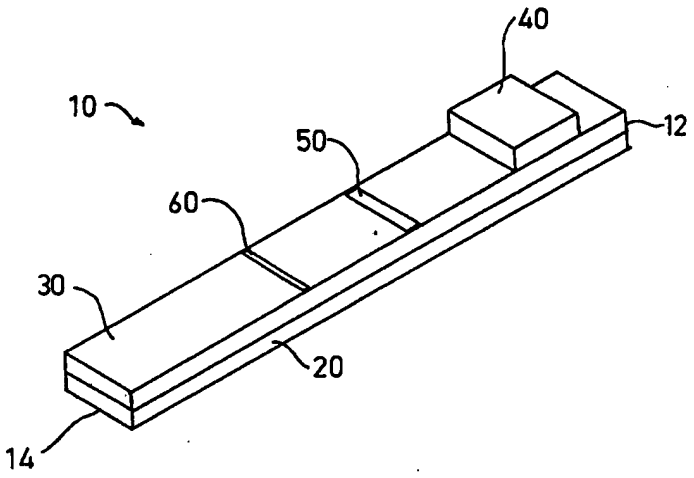

(57) Abstract

The invention relates to a method and test strip for detecting the presence of an analyte of interest in a biological liquid sample. The test strip comprises a porous membrane (30) adhered to a solid support (20) which has a first end (12) and a second end (14); a matrix (40) comprising labelled particles fixed to the first end of the membrane, which particles are capable of conjugating with an analyte of interest which may be present in the sample to form a conjugate which can migrate along the membrane toward the second end when the membrane is in a wet state and at least one test zone (50) on the membrane downstream of the matrix, which test zone comprises a first biological agent bound thereto which is capable of specifically conjugating with the analyte of interest, and at least one control zone (60) delineated on said membrane and located downstream of said matrix, which comprises a detectable marker capable of being washed away from the control zone on contact with said liquid sample, as said liquid sample migrates across said control zone towards said second end.

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IMMUNOCHROMATOGRAPHY TEST STRIPS

BACKGROUND OF THE INVENTION

The invention relates to an analytical test strip for the detection of an analyte of interest present in a liquid sample, based on immunochromatography of the analyte.

Several immunoassay methods for detecting antibodies or antigens are known in the art. In principle, such methods are base on the binding of an antibody or antigen of interest to an immobilized specific antigen or antibody thereagainst, or any other biological agent capable of specifically conjugating therewith to form a complex, and detecting a specific signal, which indicates the formation of such complex. Signals used may be radioisotopic signals (such as ^{3}H, ^{125}I, ^{57}Co), enzymatic signals (such as horseradish peroxidase, alkaline phosphatase), luminescent signals (such as luminol or acridine) or immunochromatographic signals (such as colloidal gold or colored latex particles).

More specifically, immunochromatography methods known in the art are based on conjugation of antigens or antibodies to the colored particles to form conjugates. Antigens or antibodies, directed against the analyte of interest, referred to as "capturers", are bound to a porous membrane, optionally fixed onto a solid support, at a specific line along the membrane, referred to as the test line. According to this methodology, a liquid sample containing the analyte of interest to be detected is applied to one end of the test strip which contains the colored particles. As a result, the sample and the colored particles migrate together along the porous membrane. In case the sample contains the analyte of interest, such analyte, when applied to the membrane, will conjugate to part of the colored particles and move along the porous membrane until reaching the test line containing the "capturers". The analyte will conjugate

with its "capturer", forming a complex which would not further migrate along the porous membrane, fixing thereto also part of the colored particles, thus producing a visible colored signal, usually a fixed line, indicating the presence of the analyte of interest in the liquid sample, a positive result. Any unconjugated colored particles will continue to migrate along the porous membrane until reaching a control line, comprising, bound thereto, the analyte of interest already conjugated to its "capturer". As a result, the particles will bind to the bound analyte and a visible control line will also appear. In case the analyte of interest is absent from the tested sample, the unconjugated colored particles will pass through the test line, until reaching the control line. As a result, only the visible control line will appear. Since at the position of the test line no visible color has been formed, the appearance of only the single, control line will indicate a negative result.

Typical immunochromatographic tests for measuring antigen are for detection of Human Chorionic Gonadotropin (HCG) and Human Immunodeficiency Virus (HIV). In the latter, antibodies present in the sample, bound to protein A conjugate with colloidal gold and this conjugate will bind, in a positive test, to the HIV antigens, fixed onto the membrane in the test line.

SUMMARY OF THE INVENTION

The present invention relates to a test strip for detecting the presence of an analyte of interest in a biological liquid sample, said strip comprising a porous membrane suitably adhered to a solid support and having a first end and a second end; a matrix comprising labelled particles suitably fixed onto said first end of said porous membrane, said labelled particles capable of conjugating with said analyte of interest, upon contact therewith, to form a conjugate capable of migrating at least downstream along said membrane toward said second end when said membrane is in a wet state; at least one test zone

delineated on said porous membrane downstream of said matrix, said at least one test zone comprising a first biological agent bound thereto capable of specifically conjugating with said analyte of interest, at least one control zone delineated on said membrane and located downstream of said matrix, said at least one control zone comprising a coloured material capable of being washed away from said control zone on contact with said liquid sample, as said liquid sample migrates across said control zone towards said second end.

The test strip according to the invention may comprise a single test zone downstream of said matrix, or a plurality of test zones preferably, also downstream to said matrix.

Within the test strip of the invention, said coloured material is chemically inert with respect to at least the analyte of interest, and said labelled particles are bound to a second biological agent.

The strip of the invention may be accommodated within a housing, said housing enabling at least part of said matrix to be in communication with the exterior of said housing such that said sample can be applied to said test strip, and further comprising a window juxtaposed over at least a portion of said strip, the said membrane located on said portion being in visual communication with the exterior of the housing, wherein said portion comprises at least a part of at least one said test zone and further comprises said at least control zone.

In addition the invention is concerned with the different methods for detecting the presence of an analyte of interest in a biological liquid sample employing the test strip of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates a perspective view of the first embodiment of the test strip according to the invention;

Figure 2 illustrates a perspective view of the second embodiment of the test strip according to the invention;

Figure 3a-3e illustrates a plan view of the test strip with different configurations of the test zones; (a) a single test zone being in the form of a narrow band; (b) a single test zone in the form of an elongated band; (c) a test strip comprising a plurality of test zones, each in the form of a narrow band and sequentially located adjacent one to another; (d) the test strip of Figure 3(c) comprising a low quantity of the analyte of interest, thus exhibiting only one signaled test zone; (e) the test strip of Figure 3(c) comprising a high quantity of the analyte of interest, thus exhibiting a plurality of signaled test zones.

Figure 4a-4b Figure 4(a) illustrates a plan view of one embodiment of a housing accommodating the test strip of Figure 2; Figure 4(b) is a cross-sectional view along the line C-C of Fig. 4(a).

Figure 5a-5b Figure 5(a) illustrates a plan view of a different embodiment of a housing accommodating the test strip of Figure 2; Figure 5(b) is a cross-sectional view along the line D-D of Fig. 5(a).

Figure 6a-6c Figure 6(a) illustrates a fragmented plan view of the housing showing the strip of Figure 3(b) via the window of the housing; Figure 6(b) illustrates schematically the manner in which a relatively low amount of analyte is detected; Figure

6(c) illustrates schematically the manner in which a relatively high amount of analyte is detected.

Figure 7a-7d Figure 7(a) illustrates a fragmented plan view of the housing showing a strip, comprising a test zone in the form of a triangle, via the window of the housing; Figures 7(b) to 7(d) illustrate schematically the manner in which positive results are indicated in relation to the appearance of the signaled triangle.

Figure 8a-8d Figure 8(a) illustrates a fragmented plan view of the housing showing the strip of Figure 3(c) via the window of the housing; Figure 8(b) illustrates a negative result; Figure 8(c) illustrates a positive result when a low amount of analyte is present in the sample; Figure 8(d) illustrates a positive result however when a high amount of analyte is present in the sample.

Figure 9a-9c Figure 9(a) illustrates a fragmented plan view of the housing showing the strip of Figure 3(a), further comprising an auxiliary test zone, via the window of the housing; Figure 9(b) illustrates a negative result; Figure(c) illustrates a positive result.

Figure 10 illustrates a test strip comprising an extended support and an observation cover according to the third embodiment of the invention.

Figure 11a-11e Figure 11(a) illustrates one example of a control zone as viewed through the observation cover of Figure 10; Figure 11(b) illustrates a negative result obtained with the strip of Figure 10; Figure 11(c), Figure 11(d) and Figure 11(e) illustrate positive results obtained with the strip of Figure 10

with a low, medium and high amount of analyte in the sample, respectively.

Figure 12 illustrates a perspective view of the fourth embodiment of the test strip according to the invention;

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a test strip for detecting the presence of an analyte of interest in a biological liquid sample, said strip comprising a porous membrane suitably adhered to a solid support and having a first end and a second end; a matrix comprising labelled particles suitably fixed onto said first end of said porous membrane, said labelled particles capable of conjugating with said analyte of interest, upon contact therewith, to form a conjugate capable of migrating at least downstream along said membrane toward said second end when said membrane is in a wet state; at least one test zone delineated on said porous membrane downstream of said matrix, said at least one test zone comprising a first biological agent bound thereto capable of specifically conjugating to said analyte of interest, at least one control zone delineated on said membrane and located downstream of said matrix, said at least one control zone comprising a coloured material capable of being washed from said control zone on contact with said liquid sample, as said liquid sample migrates across said control zone towards said second end.

By way of example only, some preferred embodiments of this invention are described hereinbelow in detail, with reference to the accompanying drawings.

1st Embodiment

Figure 1 illustrates the first embodiment of a typical strip for detecting the presence of an analyte of interest in a biological liquid sample according the

present invention. According to this embodiment, the strip of the invention, generally designated (10), comprises a porous membrane (30) suitably adhered to a solid support (20). The strip (10) is typically elongate and conveniently rectangular, and comprises a first end, (12) and a second end, (14), wherein said first end is upstream to said second end. The strip (10) is also preferably of a rectangular cross section, having a width greater than its depth. Typically, said membrane is a cellulose or nitrocellulose membrane. Cellulose membranes can be pretreated by various methods, for example by cyanogen bromide activation for photoactivation reagents. Further, the membrane can be further coated with a blocking reagent which is capable of preventing a false positive result.

A matrix (40), comprising labelled particles, is suitably fixed onto said first end (12) of said porous membrane (30). Said labelled particles are capable of conjugating with an analyte of interest, upon contact therewith, to form a conjugate which is capable of migrating at least downstream along said membrane toward said second end (14) when said membrane (30) is in a wet state. The said labelled particles are typically colloidal gold particles or alternatively coloured latex particles and are thus readily visible to the user. The labelled particles are preferably bound to a second biological agent which may be a protein or a peptide, preferably protein A or an antibody directed against immunoglobulin A. Alternatively, the labelled particles may be bound to other biological or chemical agents which in the presence of said analyte of interest may lead to the production of a detectable signal. The said matrix (40) is typically comprised of fiber glass or polyester, or any other suitable material which does not prevent the labelled particles from migrating along the membrane (30) when the latter is in a wet state.

The test zone (50) of the invention is preferably delineated on said porous membrane (30) downstream of said matrix (40), and comprises a first

biological agent, bound to said porous membrane in said test zone (50), which is capable of specifically conjugating with said analyte of interest. The test zone may be in the form of a narrow band across substantially the width of said strip (10) and substantially orthogonal to the migration path of the liquid sample through the membrane (30). Nonetheless, other configurations of the test zone are possible, as is the delineation of a plurality of test zones on said strip (10), as hereinafter described for different embodiments of the present invention.

The test strip (10) further comprises at least one control zone (60) which is delineated on said membrane (30) and is typically located downstream of said matrix (40). In this embodiment, the control zone (60) is also located downstream of the said test zone (50), though the control zone (60) may alternatively be located upstream of the said test zone (50) or within it. Having said control zone within said test zone may be advantageous, for example because it enables the user to anticipate the zone in which a positive result signal, if any, would appear. The control zone (60) comprises a coloured material capable of being washed away from said control zone (60) upon contact with said liquid sample, as said liquid sample migrates across said control zone towards said second end. The said coloured material is chemically inert with respect to at least the analyte of interest. By the term 'inert' it is meant that the coloured material does not react with the analyte of interest and does not interfere with its capability to conjugate with said first biological agent in said test zone. Said coloured material is typically a food dye, a blue cobalt salt or any other suitable chemically inert water-soluble marker.

The test strip (10) may be used as follows. A biological or chemical liquid sample is brought into contact with the matrix (40) by pipetting the sample directly onto the membrane, for example, or alternatively by immersing at least a portion of the strip,. preferably at the first end (12) of said strip (10), and

more preferably at least including a portion of the matrix (30), in a quantity of the liquid sample which may be contained in a holding vessel. The liquid sample is absorbed into the matrix (40) and from there into the membrane (30) (which as result becomes wet), together with said labelled particles which comprise said second biological agent. The migration path of the liquid sample is generally parallel to the length of the strip.

If the analyte of interest is present in the liquid sample, the labelled particles will conjugate therewith to form a conjugate which migrates along said membrane, and which in turn conjugates with the said first biological agent bound to membrane in the test zone (50) and becomes immobilised. As a result, the test zone (50) contains a signal produced by the analyte-labelled particles (with second biological agent)-first biological agent complex which is immobilised within said test zone, which signal is detectable by the user. A typical signal would be the appearance of a colour at the said test zone (50).

Thus, as the liquid sample, together with the labelled particles, migrate downstream toward the said second end (14) of the strip (10), the coloured material comprised in the control zone is washed away, thereby conclusively indicating to the user that the strip is functioning normally. If the analyte of interest in not present in the sample, the labelled particles migrate together with the sample towards the second end (14), past the said one or more test zones (50).

2nd Embodiment

Figure 2 illustrates the strip (110) according to a second embodiment of the present invention. The strip (110) comprises a porous membrane (130) on a backing (120), and a matrix (140), with at least one test zone (150) and a control zone (160), and is substantially similar to the strip (10) hereinbefore

described in relation to the first embodiment, *mutatis mutandis*. In the second embodiment, the strip (110) further comprises a first bibulous or spongy material (170), which may be pretreated with a suitable buffer solution suitably fixed onto at least said matrix., and optionally a second bibulous or spongy material (180) suitably fixed to said porous membrane downstream of said control zone and said test zone. Said first spongy material may also be fixed onto part of said membrane (130), and may act as a reservoir for receiving the liquid sample and releasing it onto at least the matrix (130), and optionally also directly onto the membrane (130). The second spongy material (180) may act as a reservoir to collect the liquid sample, together with washed-away coloured material and/or labelled particles after migration of the liquid sample through the said membrane (130), past the said control zone (160).

Preferably, said first spongy material and said second spongy material is filter paper, and said buffer is PBS (phosphate buffered saline), borate buffer or any other suitable buffer.

The said strip (110) according to the second embodiment of the present invention may comprise a single test zone (150) downstream of said matrix (140), said test zone (150) being in the form of a narrow band (151) across substantially the width of said strip and substantially orthogonal to the migration path of said liquid sample on said membrane (130), as illustrated in Figure 3(a). In general, such a test zone configuration is useful for the qualitative detection of an analyte of interest in a liquid sample.

Alternatively, the single test zone (150) may be in the form of an elongate band (152) having a width substantially equal to the width of the said strip (110), and is illustrated in Figure 3(b). In general, such a test zone configuration may give an indication to the user of the quantity of analyte in

the sample: the greater the quantity of analyte, the larger the area of the test zone that is rendered detectable.

In general, the development of a quantifiable signal in the test zone may be assessed with an appropriate form of instrumentation.

Alternatively, the test strip (110) may comprise a plurality of test zones (150). These test zones (150) may be in the form of a narrow bands (153) across substantially the width of said strip (110), sequentially located, preferably adjacent one to another on said strip (110), as illustrated in Figure 3(c). In general, such a test zone configuration may give an indication to the user of the quantity of analyte in the sample in a digital-type display. The greater the quantity of analyte, the larger the number of bands (153) that are rendered visible. Figures 3(d) and 3(e) illustrate schematically the manner in which positive results may be indicated on such a strip when analyte is present in the sample in relatively low and high quantities, respectively.

The strip (110) of the second embodiment may be used in a similar manner to that described for the strip (10) of the first embodiment, *mutatis mutandis*, with the difference that liquid sample may be absorbed into the matrix (140) via the first spongy material (170).

Optionally, the strip (110) may be accommodated within a housing (100), said housing (100) enabling at least part of said matrix (140) to be in direct or indirect communication with the exterior of said housing (100) such that said sample can be applied to said test strip (110). In one embodiment of the housing (100), illustrated in Figure 4, the said matrix (140) is in communication with the exterior (600) of said housing (100) via an inlet port (200) comprised therein, wherein said inlet port (200) enables said sample to be brought into contact with said matrix (140) via said first spongy material (170). Typically, a sample of liquid may be pipetted onto said first spongy

material (170) through said inlet port (200). Alternatively, said matrix (140) is in communication with the exterior (600) of said housing (100) via a bibulous receiving member (500), as illustrated in Figure 5. The receiving member (500) protrudes from said housing (100) via a suitable opening (550) upstream of said matrix (140) and is in communication therewith. Typically, said receiving member (500) is an upstream extension of said membrane and support (120) past said first end (112), and may further comprise a parallel upstream extension of said first spongy material (170). The receiving member (500) may act as a reservoir for receiving said liquid sample and releasing it onto at least said matrix (140). Typically, a biological liquid sample is brought into contact with said receiving member (500) by immersing at least the upstream end of the same into a vessel comprising a quantity of said liquid sample.

Typically, said housing (100) comprises a hollow casing construction, and is made from a moisture impervious solid material such as a suitable plastic material, for example.

The housing (100) further comprises a window (300) juxtaposed over at least a portion (115) of said strip (110), so that the membrane (130) located on said portion (115) is in visual communication with the exterior (600) of the housing (100). The said portion (115) comprises at least a part of at least one said test zone (150) and further comprises said control zone (160). Said window is typically rectangular in form, preferably having a width slightly narrower than that of the strip (110).

When said strip (110) comprises a test zone (150) in the form of an elongate band (152) as hereinbefore described, the said casing (100) may further comprise a suitable scale (111) along at least one side of said window (100) parallel to the migration path of the liquid, as illustrated schematically in

Figure 6(a). The scale (111) may be calibrated to provide the user with a measure of quantity of analyte in the sample. Figure 6(b) and 6(c) illustrate schematically the manner in which positive results may be indicated on such a strip when analyte is present in the sample in relatively low and high quantities, respectively.

Alternatively, the said strip (110) may comprise test zone (150) in the form of an elongate triangle (156) having its base at the downstream end of the window and its apex at the upstream thereof, as illustrated in Figure 7(a). Figure 7(b) and 7(c) illustrate schematically the manner in which positive results may be indicated on such a strip when analyte is present in the sample in relatively low and high quantities, respectively.

Alternatively, the said strip (110) may comprise a plurality of test zones in the form of narrow bands (153), as illustrated schematically in Figure 8(a). Figure 8(b) illustrates schematically a negative result for this arrangement, while Figures 8(c) and 8(d) illustrate schematically the manner in which positive results may be indicated on such a strip when analyte is present in the sample in relatively low and high quantities, respectively. Optionally, a suitable scale may be provided on the said housing adjacent to the window (300), in a similar manner to scale (111) hereinbefore described.

Alternatively, and with reference to Figure 9(a), said strip (110) further comprises at least one auxiliary test zone (157) upstream of said window (300) comprising said first biological agent in sufficient amount for conjugating with a quantity of said analyte in said sample up to a predetermined minimum quantity of said analyte. Thus if a liquid sample contains the analyte of interest in quantities equal to or lower than said minimum quantity, all of the analyte will conjugate with the said first biological agent (bound to said labelled particles), bound to said auxiliary test zone (157). In such circumstances, a

negative result will be indicated to the user, since the auxiliary test zone (157) is preferably obscured by the casing and the positive result on the auxiliary test zone (157) will thus not be visible to the user, as illustrated in Figure 9(b). When the analyte of interest is present in said sample in quantities in excess of said minimum quantity, the first biological agent in said auxiliary test zone (157) is conjugated with said analyte to saturation, allowing the excess analyte to conjugate with said biological agent bound in said test zone (150) comprised within said portion (115), rendering the said test zone (150) at least partially detectable by the user, as illustrated in Figure 9(c). In this manner, it is possible to calibrate the quantity of first biological agent in said auxiliary testy zone (157) for tests where only the detection of quantities of analyte greater than a predetermined minimum quantity is of interest to the user.

The said housing (100), may alternatively accommodate the strip (10) of the said first embodiment in a similar manner to the second embodiment of the strip of the present invention, *mutatis mutandis*.

3rd Embodiment

A third embodiment of the present invention is illustrated in Figure 10. In this embodiment the test strip (700) for detecting the presence of an analyte of interest according to the present invention comprises a porous membrane (730) suitably attached to a solid support (720) at a first end (712) thereof. The said membrane (730) is typically in the form of a rectangle of low aspect ratio, having a width approximately the same as its length and is preferably a cellulose or nitrocellulose membrane. The said support is typically of a width substantially similar to that of the membrane (730), but comprises a much greater length, such that the second end (714) of the support (720) is free of the membrane (730) and may be used as a handle by a user. The strip (700) further comprises an observation cover (710), typically made from a suitable

transparent material, suitably attached to the said membrane (730). In this embodiment, the cover (710) has substantially the same size and shape as the membrane (730) and is juxtaposed on the same. The four edges (735) of the membrane (730) are thus left exposed. A control zone (760) may be delineated on the said membrane (730), comprising a coloured material capable of being washed away by the liquid sample on contact therewith. The control zone (760) may be in the form of a ring, for example, as illustrated in Figure 11(a), or in any other suitable form. The strip (700) further comprises a test zone (750) delineated on the membrane (730) comprising a first biological agent bound thereto capable of specifically conjugating to said analyte of interest. In this embodiment the test zone (750) extends across substantially the full width and length of the membrane (730), as illustrated in Figure 11(b).

The strip (700) of the present invention may be used as follows. The said first end (712) of the strip (700) is immersed into a vessel containing the said biological liquid sample, previously mixed with labelled particles, so that the free edges (735) of the membrane (730) are swelled by the liquid mixture. Alternatively, the strip may be immersed sequentially in two different vessels, one containing the liquid sample to be tested and the other containing the labelled particles in a suitable buffer solution. The strip is then removed from the vessel(s). The disappearance of the said colour from said control zone (760) indicates that the test is valid and that the strip was used properly, while there are no other detectable changes in the test zone (760), indicates a negative result. Figures 11(c), 11(d) and 11(e) illustrate schematically the manner in which positive results may be indicated on such a strip when analyte is present in the sample in relatively low, medium and high quantities, respectively. Thus, this embodiment is useful in determining the presence of analyte in both a quantitative and qualitative manner.

4th Embodiment

Figure 12 illustrates the fourth embodiment of a typical strip for detecting the presence of an analyte of interest in a biological liquid sample according the present invention. The strip, generally designated (800) comprises a porous membrane (830) suitably adhered to a solid support (820). The strip (800) is typically elongate and conveniently rectangular, and comprises an first end (812) and a second end (814). The strip (800) is also preferably of a rectangular cross section, having a width greater than its depth. Typically, said membrane (830) is a cellulose or nitrocellulose membrane.

The said strip (800) comprises a test zone (850) of the invention is preferably delineated on said porous membrane (830) and comprises a first biological agent, bound in said test zone (850) to said porous membrane capable of specifically conjugating with said analyte of interest. The test zone (850) is preferably in the form of an elongate band (852), though it may take other forms.

The strip (800) according to the invention further comprises at least one control zone (860) which is delineated on said membrane (830) and typically located downstream of the said test zone (850), though the control zone (860) may alternatively be located upstream of the said test zone (850) or within it. The control zone (860) comprises a coloured material capable of being washed away from said control zone (860) upon contact with said liquid sample. The said coloured material is chemically inert with respect to at least the analyte of interest, and said coloured material is typically a food dye, a blue cobalt salt or any other suitable chemically inert water-soluble marker.

The test strip (800) of the invention may be used as follows. The said first end (812) of the strip (800) is immersed in a biological liquid sample contained in a vessel, previously mixed with labelled particles. Alternatively, the strip may

be immersed sequentially in the liquid sample to be tested contained in one vessel and in a buffer solution containing the labelled particles contained in another vessel in a suitable buffer solution. The strip is then removed from the vessel(s). The disappearance of the colour from the said control zone (860) indicates that the test is valid and that the strip was used properly, while there are no other detectable changes in the test zone (860), indicates a negative result. Whereas, with a positive result, a detectable signal, distinguishable from the coloured marker will appear in said test zone.

According to the present invention, and within the preferred embodiments as hereinbefore described, said labelled particles are preferably bound to a second biological agent, said second biological agent being a protein, a peptide, or an antibody directed against a human immunoglobulin.

The analyte of interest to be detected can be an antibody, such as antibodies directed against Human Immunodeficiency Virus 1 (HIV-1), Human Immunodeficiency Virus (HIV-2), Hepatitis B surface Antigen (HBsAG) or Hepatitis C Virus (HCV), such that the said first biological agent comprises antigens which specifically bind to said HIV-1, HIV-2, HBsAg or HCV antibodies, respectively.

The analyte of interest can also be a hormone, such as, for example, Human Chorionic Gonadotropin (HCG), Luteinizing Stimulating Hormone (LH), Follicle Stimulating Hormone (FSH) or Thyroid Stimulating Hormone (TSH), and said first biological agent thus is preferably selected from antibodies which specifically bind to said HCG, LH, FSH or TSH, respectively.

Further, the analyte of interest may be CK-MB, prolactine, an opiate, THC or an amphetamine, and thus said first biological agent may comprise antibodies which specifically bind to said CK-MB, prolactine, opiate, THC or amphetamine, respectively.

Further, the analyte of interest may be any other biological or chemical material. Thus, other proteins or peptides may be detected by the strip of the invention. Such proteins may be, for example, enzymes which are capable of conjugating with said labelled particles and with a suitable substrate at said test zone. The action of the enzyme on the substrate may give rise to the formation of a detectable signal.

The analyte of interest may also be a microorganism, such as, for example, a *Salmonella* sp., a *Chlamydia* sp., a *Streptococcus hemoliticus* group A (Strep-A) or *Streptococcus hemoliticus* group B (Strep-B), said test zone carries antibodies directed against said *Salmonella* sp., a *Chlamydia* sp., a *Streptococcus hemoliticus* group A (Strep-A) or *Streptococcus hemoliticus* group B (Strep-B), respectively, and said first biological agent may then comprise antibodies which specifically bind to said *Salmonella* sp., *Chlamydia* sp., Strep-A or Strep-B, respectively.

Of particular interest, but not limited thereto, the analyte to be detected in said biological liquid sample is HCG.

In a different aspect, the invention relates to a method for detecting the presence of an analyte of interest in a biological liquid sample comprising the steps of (a) providing a test strip comprising: (i) a porous membrane suitably adhered to a solid support; (ii) at least one test zone on said porous membrane comprising a first biological agent bound thereto, capable of specifically conjugating to said analyte of interest; (iii) at least one control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone on contact with said liquid sample; (b) bringing said biological liquid sample into contact with labelled particles bound to a second biological agent, said second biological agent being capable of conjugating with said analyte of interest, upon contact

therewith; (c) immersing the test strip obtained in step (a) in the mixture obtained in step (b), such that said analyte of interest, if present in said biological liquid sample, specifically conjugates with said first biological agent; whereby the mere disappearance of said coloured material from the control zone indicates the absence of said analyte from said sample and the disappearance of said coloured material from the control zone together with the appearance of a detectable signal indicative of the presence of said labelled particles in said test zone indicates the presence of analyte in said sample.

Further, a test strip for use in the above described method of the invention, is within the scope of the invention. This test strip comprises a porous membrane suitably adhered to a solid support; at least one test zone on said porous membrane comprising a first biological agent bound thereto, capable of specifically conjugating with said analyte of interest; and at least one control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone upon contact with said liquid sample.

In an additional embodiment, the invention provides a test strip for use in the method of the invention as hereinbefore described, which comprises a porous membrane suitably adhered to a solid support, said solid support substantially extending upstream from said membrane to provide a handle-like extension; a test zone on said porous membrane comprising a first biological agent bound thereto, capable of specifically conjugating with said analyte of interest; a control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone upon contact with said liquid sample, said test strip being characterized in that it further comprises a transparent, non-permeable, observation cover suitably attached onto said membrane, juxtaposed to said support, and having substantially the size and shape of said membrane.

Finally the invention is concerned with a method of detecting the presence of an analyte of interest in a biological liquid sample by employing the test strip of the invention, said method comprising the steps of applying a sufficient amount of said biological liquid sample onto said matrix; allowing the liquid sample to migrate at least downstream along said membrane, towards said second end, and determining whether or not said analyte of interest is present in said biological liquid sample whereby the mere disappearance of said coloured material from the control zone indicates absence of said analyte from said sample while the disappearance of said coloured material from the control zone together with the appearance of a detectable signal in said test zone indicates the presence of said analyte of interest in said sample and the preservation of said coloured material in said control zone indicates improper use.

The test strips of the invention may be prepared by a method comprising the following steps: adhering a porous membrane onto a solid support, applying said first biological agent (e.g. an antibody or antigen or any other biological agent which may specifically bind to said analyte of interest), preferably present in a buffer solution, onto said membrane, to form the test zone and drying the same, applying said coloured material onto said membrane to form said control zone, applying the labelled particles, preferably with said second biological agent bound thereto, to the matrix and fixing the same preferably to said first end of said porous membrane, and drying the same.

As already noted above, the coloured control zone is necessary in order to determine whether the test was conducted properly. There are, nevertheless, additional advantages of having said coloured control zone, over the test strips known in the prior art, may be found when the control zone is located within said test zone. Such an integral zone may be obtained by mixing the coloured material and the first biological agent prior to application thereof onto said

membrane. One advantage obtained thereby, for example, is significant during the process for manufacturing said test strips, since only one application process onto said membrane is required, which evidently will enable to reduce machine "printing time" by about 50%. Nonetheless, an integral test zone and control zone will enable the user to easily anticipate the location of the signal produced when the test results are positive.

In addition, the application of a mixture of a coloured material, acting as the control, with the first biological agent, will ensure the manufacturer, simply by eyesight, that the biological agent has been applied to said membrane, and thus, that the product is in integrity. Therefore, the control zone acts also s a quality control footprint.

Naturally, additional quality control means, may be provided by printing additional control zones on said membrane, in other areas. For example, a second control zone may be applied to said membrane at the second end of said strip. Such marker can easily indicate to which end of the test strip the liquid sample has to be applied, in case using the strip as such. In case the strip is accommodated within a housing which has said inlet port, if this coloured signal is visible through said inlet port, it would indicate that the strip has been inserted into said housing in the wrong direction.

Another advantage of the strip of the present invention over the known devices, usually accommodated within a housing and packed in moisture-proof packaging such as aluminum pouches, is that an accidental puncture of the packaging or production defects yielding incomplete sealing of said packaging or housing will result in a non-visible control zone. Thus, use of hygroscopic colors for the control zone, as in the present invention, will enable the indication of any exposure to humidity prior to use, since as a result of such

exposure, the colour in the control zone will change, e.g. from blue in the dry form to brown in the hydrous form.

All the embodiments of the present invention can be used for quantitative measurements when required. Any suitable instruments, as known to the man skilled in the art, may be of use, depending on the type of the detectable signal obtained. The man skilled in the art would know, in accordance with the signal produced by the presence of said analyte of interest, which methods of analytical determinations and instruments to use.

CLAIMS:

1. A test strip for detecting the presence of an analyte of interest in a biological liquid sample, said strip comprising:

   - a porous membrane suitably adhered to a solid support and having a first end and a second end;

   - a matrix comprising labelled particles suitably fixed onto said first end of said porous membrane, said labelled particles capable of conjugating with said analyte of interest, upon contact therewith, to form a conjugate capable of migrating at least downstream along said membrane toward said second end when said membrane is in a wet state;

   - at least one test zone delineated on said porous membrane downstream of said matrix, said at least one test zone comprising a first biological agent bound thereto capable of specifically conjugating with said analyte of interest,

   - at least one control zone delineated on said membrane and located downstream of said matrix, said at least one control zone comprising a coloured material capable of being washed away from said control zone on contact with said liquid sample, as said liquid sample migrates across said control zone towards said second end.

2. The test strip according to claim 1 further comprising a first spongy material which may be pretreated with a suitable buffer solution suitably fixed onto at least said matrix.

3. The test strip according to claim 1 or 2 further comprising a second spongy material suitably fixed to said porous membrane downstream of said control zone and said test zone.

4. The strip according to claim 2 wherein said first spongy material is filter paper.

5. The strip according to claim 3 wherein said second spongy material is filter paper.
6. The test strip according to any one of claims 2 to 5 wherein said buffer is PBS, borate buffer or any other suitable buffer.
7. The test strip according to any of the preceding claims comprising a single test zone downstream to said matrix, said test zone being in the form of a narrow band across substantially the width of said strip and substantially orthogonal to the migration path of said liquid sample on said membrane.
8. The test strip according to any of claims 1 to 6 comprising a single test zone downstream of said matrix, said test zone being in the form of an elongate band having a width substantially equal to the width of the said strip.
9. The test strip according to any of claims 1 to 7 comprising a plurality of test zones.
10. The test strip according to claim 9 wherein said plurality of test zones are sequentially located adjacent one to another on said strip.
11. The test strip according to any of the preceding claims wherein said porous membrane is a cellulose or nitrocellulose membrane.
12. The test strip of claim 1 wherein said control zone is located within said test zone.
13. The test strip according to claim 1 wherein said control zone is located upstream of said test zone.
14. The test strip according to claim 1 wherein said control zone is located downstream of said test zone.
15. The test strip according to any of the preceding claims wherein said labelled particles comprise colloidal gold particles or coloured latex particles.

16. The strip according to any of the preceding claims wherein said matrix is comprised of fiber glass, polyester or any other suitable material which does not prevent said labelled particles from migrating along the membrane when said membrane is in a wet state.

17. The test strip according to any of the preceding claims wherein said coloured material is chemically inert with respect to at least the analyte of interest, said coloured material being a food dye, a blue cobalt salt or any other suitable chemically inert water-soluble marker.

18. The test strip according to any of the preceding claims wherein said labelled particles are bound to a second biological agent, said second biological agent being a peptide or a protein.

19. The test strip according to any of the preceding claims wherein said analyte of interest is:

- an antibody, preferably an antibody directed against Human Immunodeficiency Virus 1 (HIV-1), Human Immunodeficiency Virus (HIV-2), Hepatitis B surface Antigen (HBsAG) or Hepatitis C Virus (HCV), wherein said first biological agent comprises antigens which specifically bind to said HIV-1, HIV-2, HBsAg or HCV antibodies, respectively;

- a hormone, preferably Human Chorionic Gonadotropin (HCG), Luteinizing Stimulating Hormone (LH), Follicle Stimulating Hormone (FSH) or Thyroid Stimulating Hormone (TSH), wherein said first biological agent comprises antibodies which specifically bind to said HCG, LH, FSH or TSH, respectively;

- CK-MB, prolactine, an opiate, THC or an amphetamine, wherein said first biological agent comprises antibodies which specifically bind to said CK-MB, prolactine, opiate, THC or amphetamine, respectively; or

- a microorganism, preferably *Salmonella* sp., a *Chlamydia* sp., a *Streptococcus hemoliticus* group A (Strep-A) or *Streptococcus hemoliticus* group B (Strep-B), said test zone carries antibodies directed

against said *Salmonella* sp., a *Chlamydia* sp., a *Streptococcus hemoliticus* group A (Strep-A) or *Streptococcus hemoliticus* group B (Strep-B), respectively, wherein said first biological agent comprises antibodies which specifically bind to said *Salmonella* sp., *Chlamydia* sp., Strep-A or Strep-B, respectively.

20. The test strip according to any of claims 1 to 19, accommodated within a housing, said housing enabling at least part of said matrix to be in communication with the exterior of said housing such that said sample can be applied to said test strip, and further comprising a window juxtaposed over at least a portion of said strip, the said membrane located on said portion being in visual communication with the exterior of the housing, wherein said portion comprises at least a part of at least one said test zone and further comprises said at least control zone.

21. The test strip according to claim 20, wherein said strip comprises at least one test zone upstream of said window for conjugating with a quantity of said analyte in said sample up to a predetermined minimum quantity of said analyte, whereby only quantities of analyte in said sample in excess of said minimum quantity are rendered visible via said window by virtue of further conjugation of said excess quantity of analyte with said biological agent comprised in a test zone comprised within said portion.

22. The test strip according to claims 20 or 21 wherein said matrix is in communication with the exterior of said housing via an inlet port comprised in said housing, wherein said inlet port enables said sample to be brought into contact with said matrix.

23. The test strip of claims 20 or 21 wherein said matrix is in communication with the exterior of said housing via a bibulous receiving member which protrudes from said housing upstream of said matrix and is in communication therewith, wherein said receiving member may act as a reservoir for receiving said liquid sample and releasing it onto at least

said matrix, said receiving member may be immersed into a vessel comprising a quantity of said liquid sample.

24. A method for detecting the presence of an analyte of interest in a biological liquid sample comprising the steps of:

- providing a test strip comprising:-
  - a porous membrane suitably adhered to a solid support;
  - at least one test zone on said porous membrane comprising a first biological agent bound thereto, capable of specifically conjugating with said analyte of interest;
  - at least one control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone upon contact with said liquid sample;
- bringing said biological liquid sample into contact with labelled particles bound to a second biological agent to form a mixture, said second biological agent capable of conjugating with said analyte of interest, upon contact therewith;
- immersing the test strip obtained in step (a) in the mixture obtained by step (b), such that said analyte of interest, if present in said biological liquid sample, specifically conjugates with said first biological agent;

whereby:

- mere disappearance of said coloured material from said control zone indicates the absence of said analyte of interest from said sample; and
- disappearance of said coloured material from said control zone together with the appearance of a detectable signal indicative of the presence of said labelled particles in said test zone indicates the presence of said analyte of interest in said sample.

25. A test strip for use in the method of claim 23, said test strip comprising:

- a porous membrane suitably adhered to a solid support;

- at least one test zone on said porous membrane comprising a first biological agent bound thereto, said first biological agent being capable of specifically conjugating with said analyte of interest;

- at least one control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone upon contact with said liquid sample.

26. A test strip for use in the method of claim 24, said test strip comprising:

- a porous membrane suitably adhered to a solid support, said solid support substantially extending upstream from said membrane to provide a handle-like extension;

- a test zone on said porous membrane comprising a first biological agent bound thereto, capable of specifically conjugating with said analyte of interest;

- a control zone delineated on said membrane, said at least one control zone comprising a coloured material capable of being washed away from said control zone upon contact with said liquid sample;

said test strip being characterized in that it further comprises a transparent, non-permeable, observation cover suitably attached onto said membrane, juxtaposed to said support, and having substantially the size and shape of said membrane.

27. A method of detecting the presence of an analyte of interest in a biological liquid sample by employing a test strip according to claim 1 comprising the steps of:

- applying a sufficient amount of said biological liquid sample onto said matrix, and allowing the liquid sample to migrate at least downstream along said membrane, towards said second end,

- determining whether or not said analyte of interest is present in said biological liquid sample whereby:

- mere disappearance of said coloured material from the control zone indicates absence of said analyte from said sample;

- disappearance of said coloured material from the control zone together with the appearance of a detectable signal in said test zone indicates the presence of said analyte of interest in said sample; and

- preservation of said coloured material in said control zone indicates improper use.

28. A method according to claim 27 or 30 for the detection of HCG in a liquid biological sample.

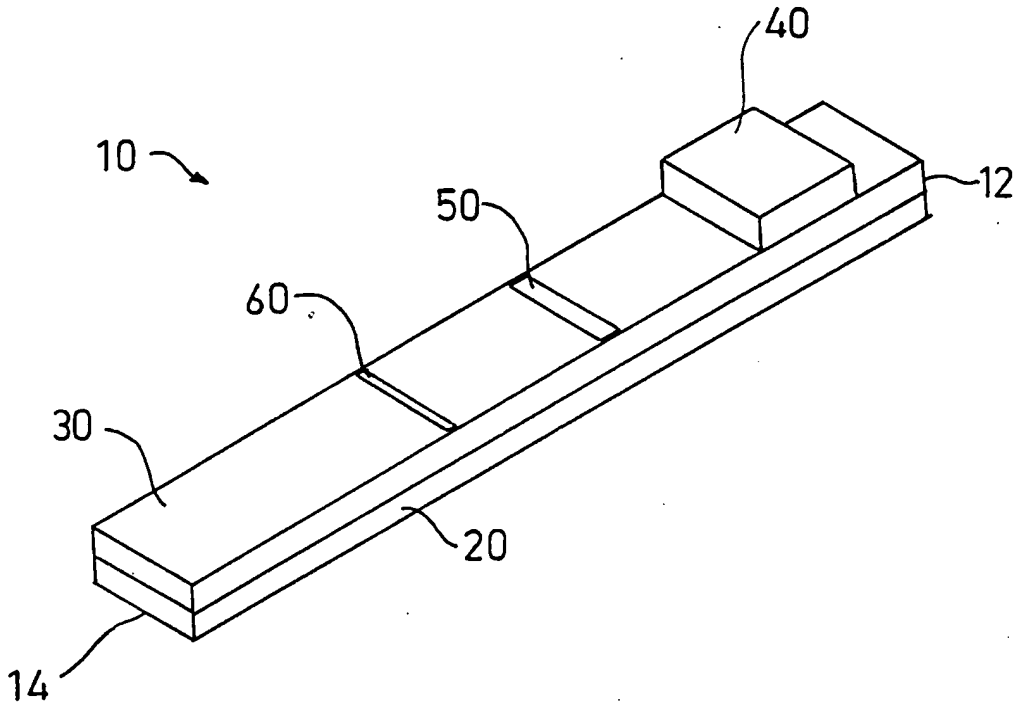

Figure 1

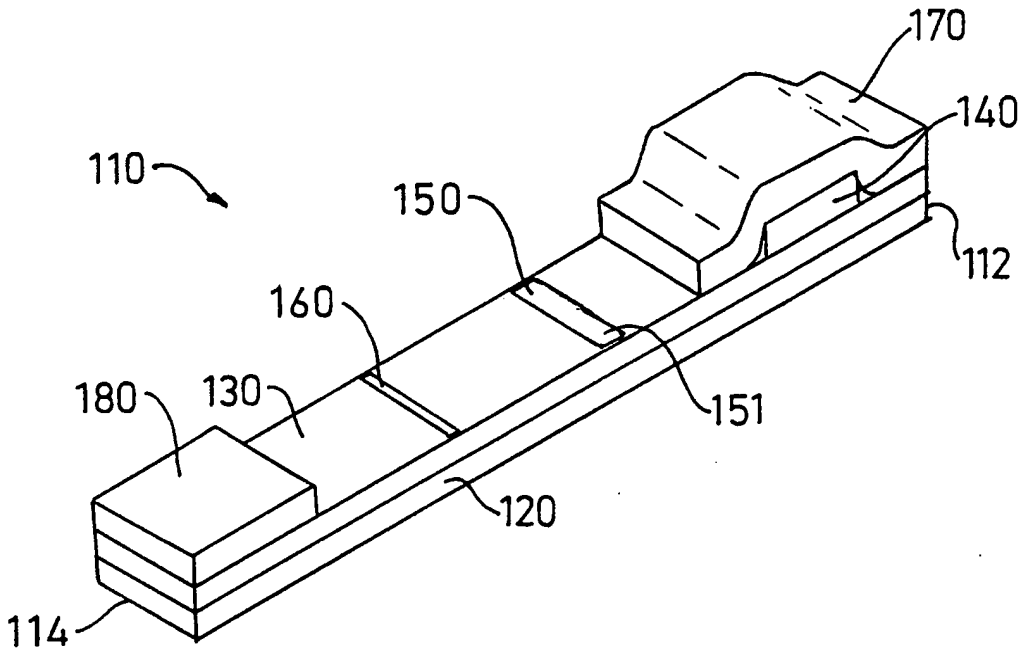

Figure 2

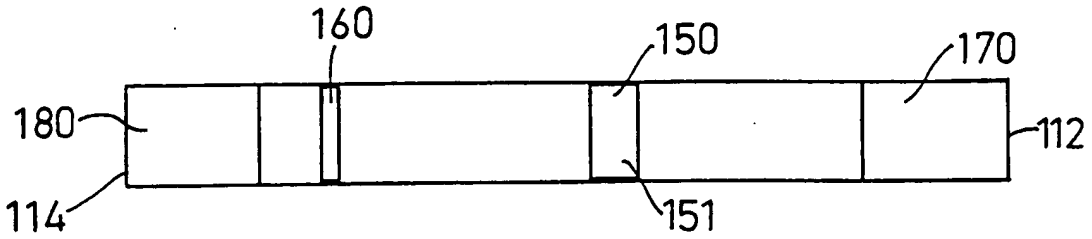

Figure 3(a)

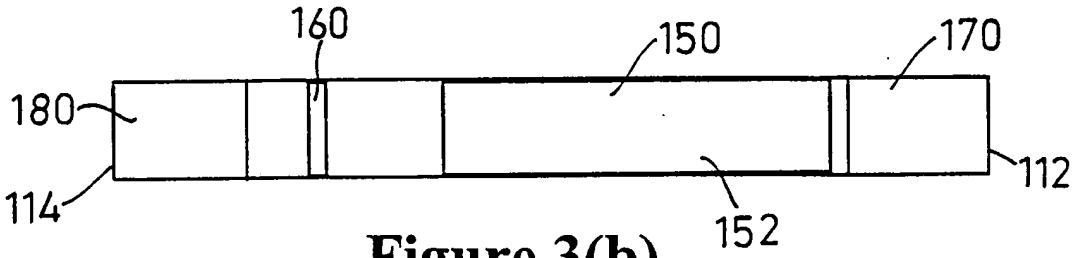

Figure 3(b)

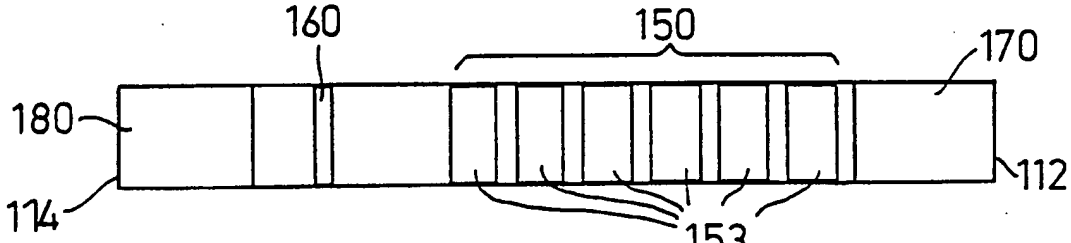

Figure 3(c)

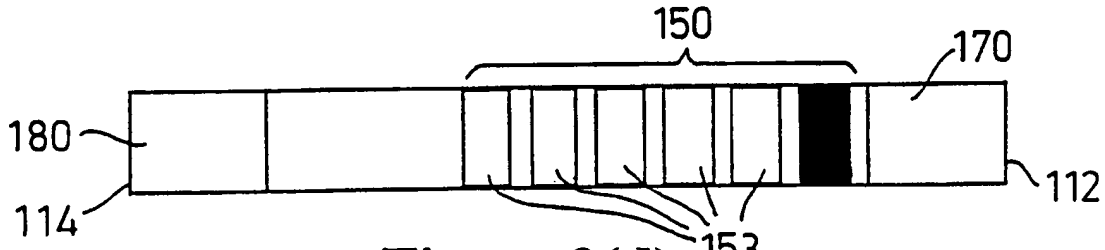

Figure 3(d)

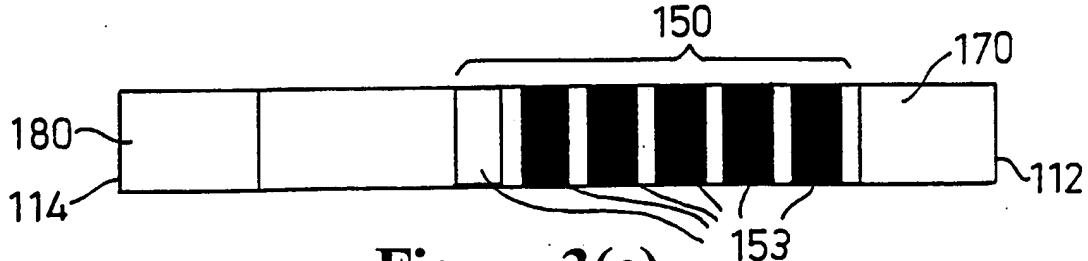

Figure 3(e)

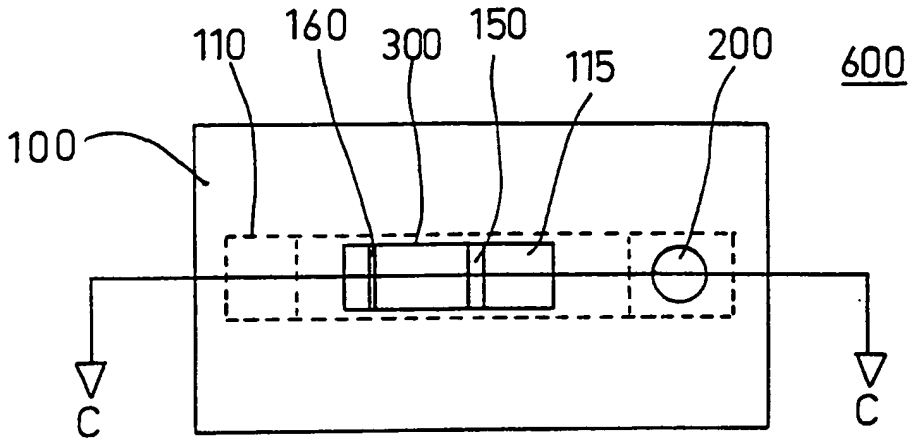

Figure 4(a)

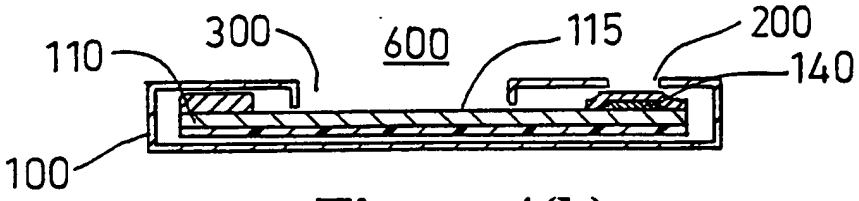

Figure 4(b)

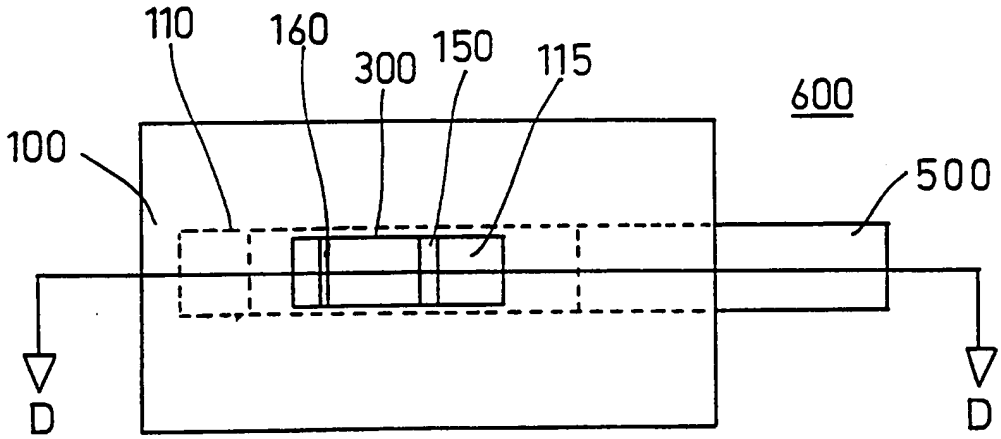

Figure 5(a)

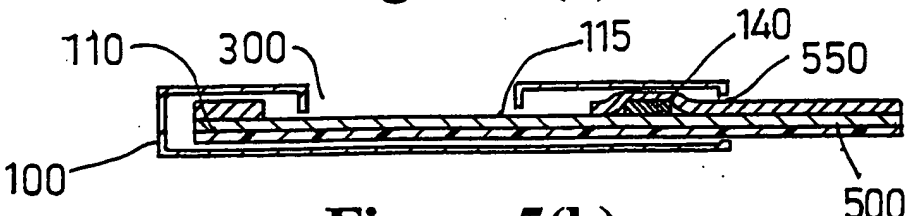

Figure 5(b)

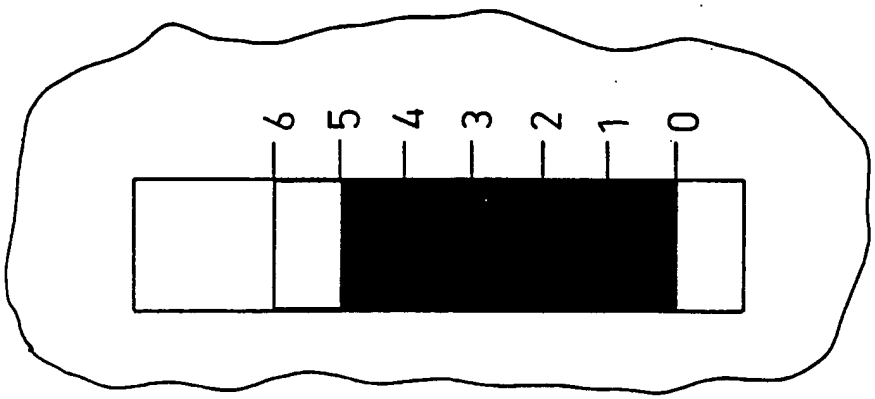

Figure 6(c)

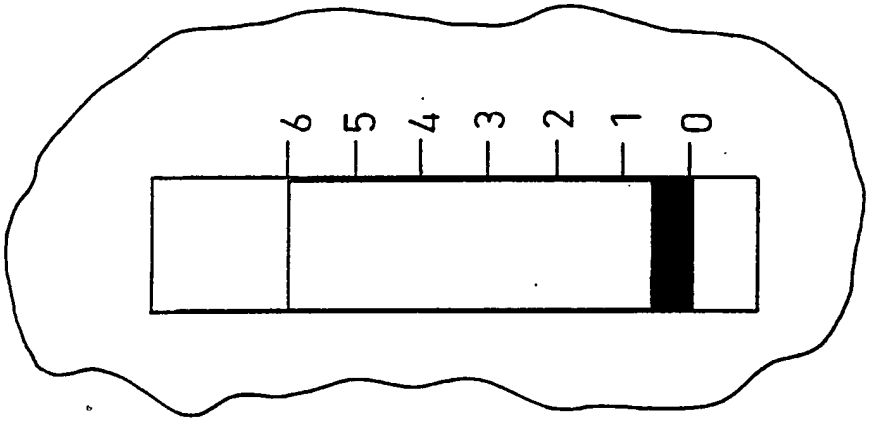

Figure 6(b)

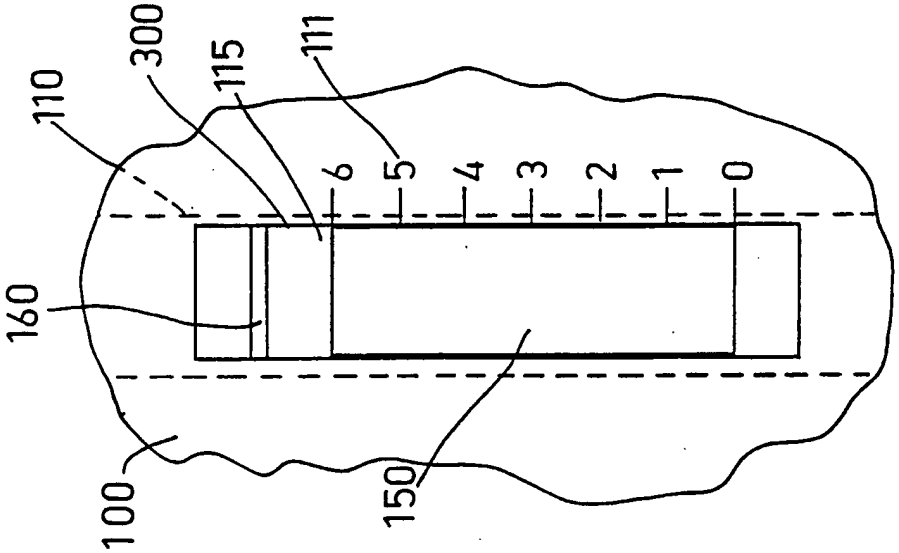

Figure 6(a)

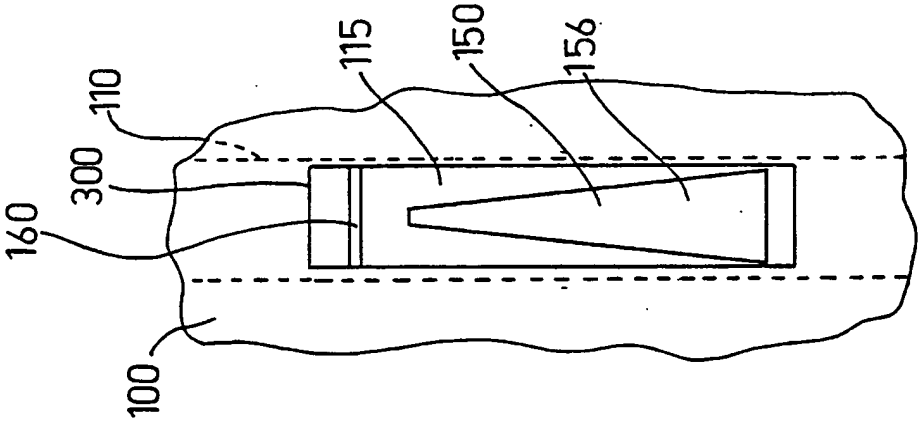

Figure 7(a)

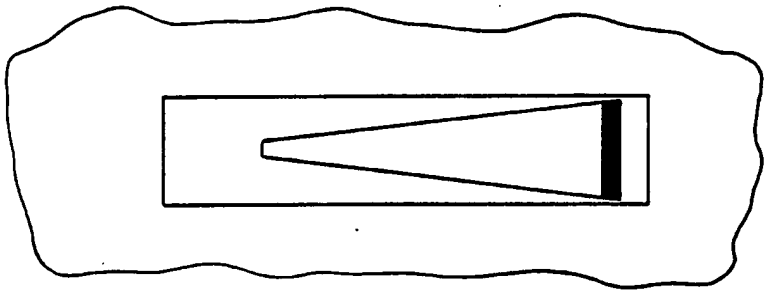

Figure 7(b)

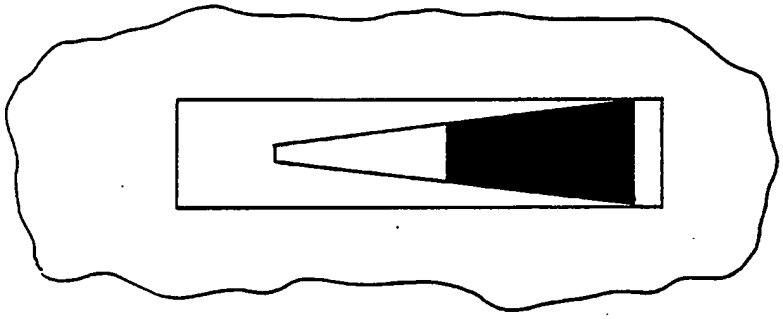

Figure 7(c)

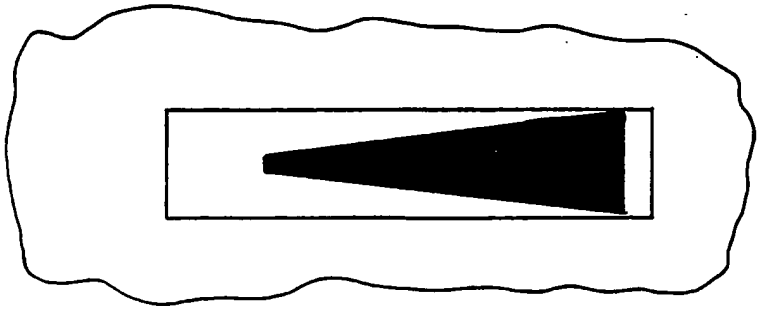

Figure 7(d)

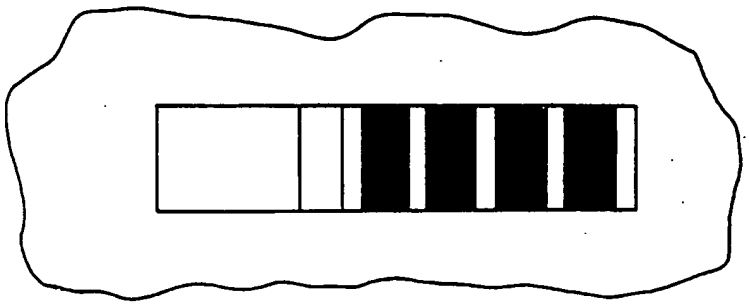

Figure 8(d)

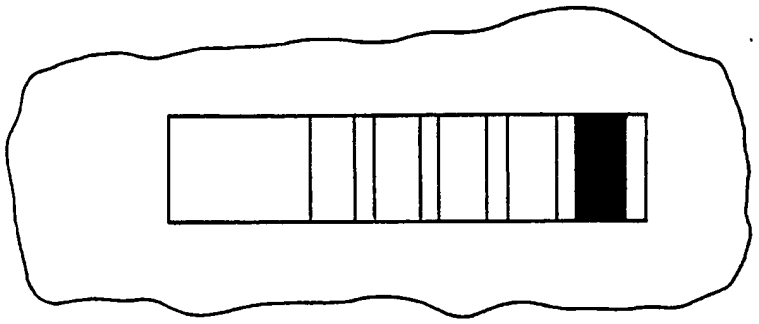

Figure 8(c)

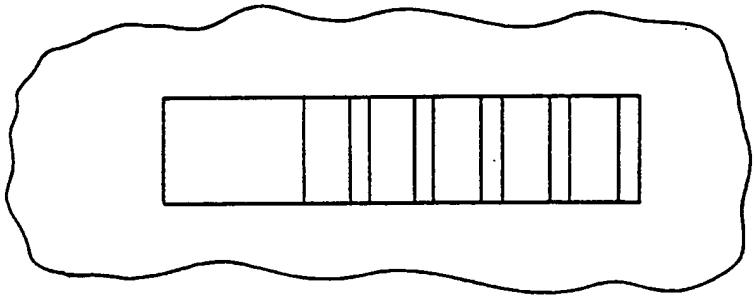

Figure 8(b)

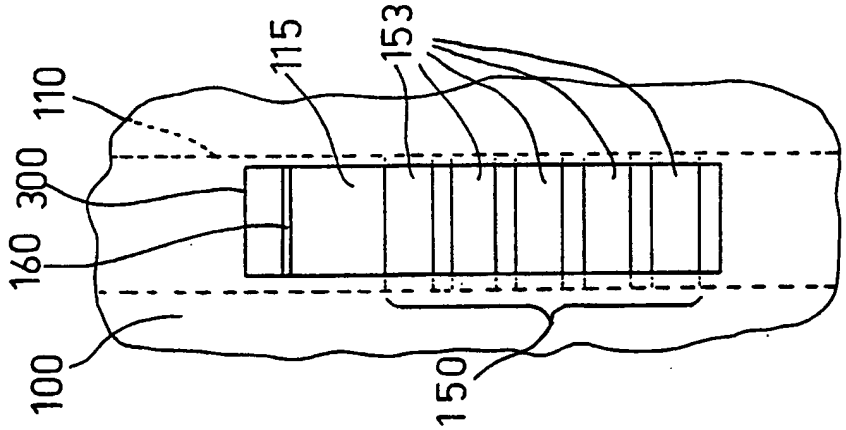

Figure 8(a)

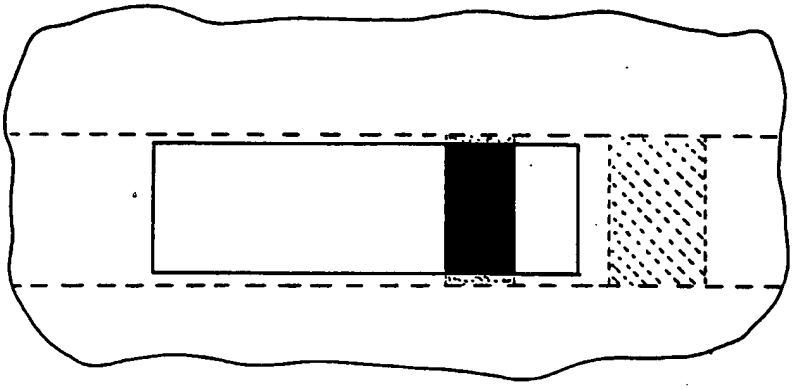

Figure 9(c)

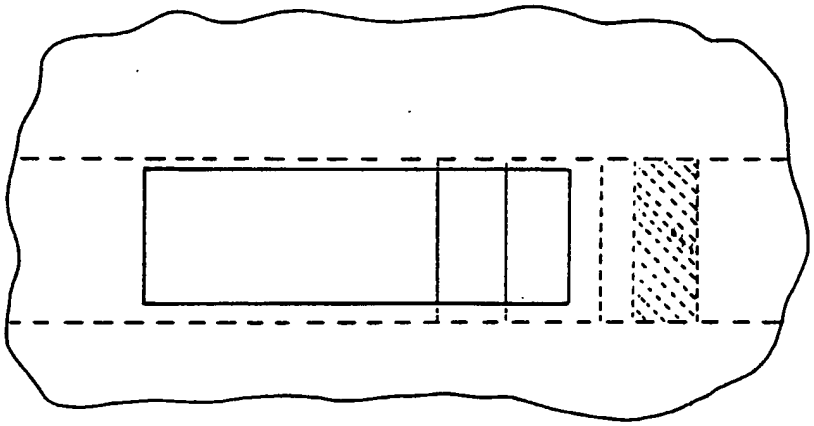

Figure 9(b)

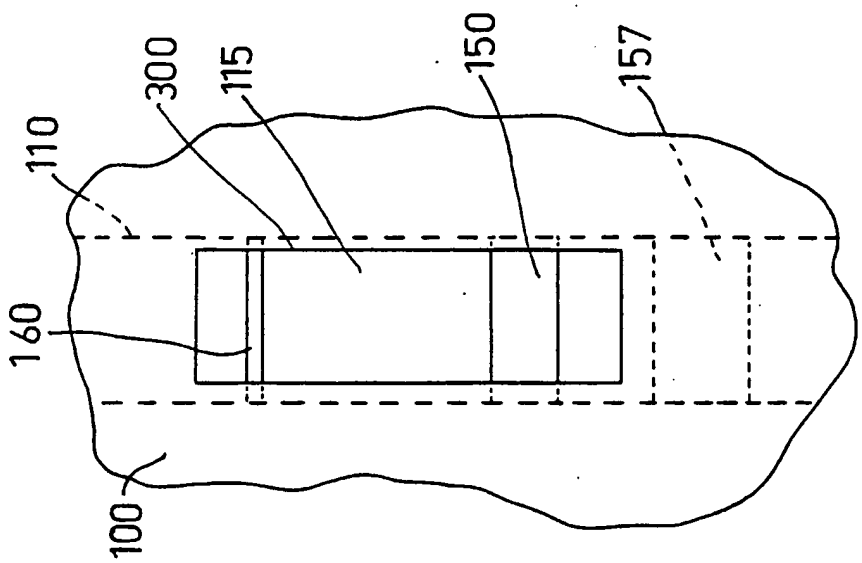

Figure 9(a)

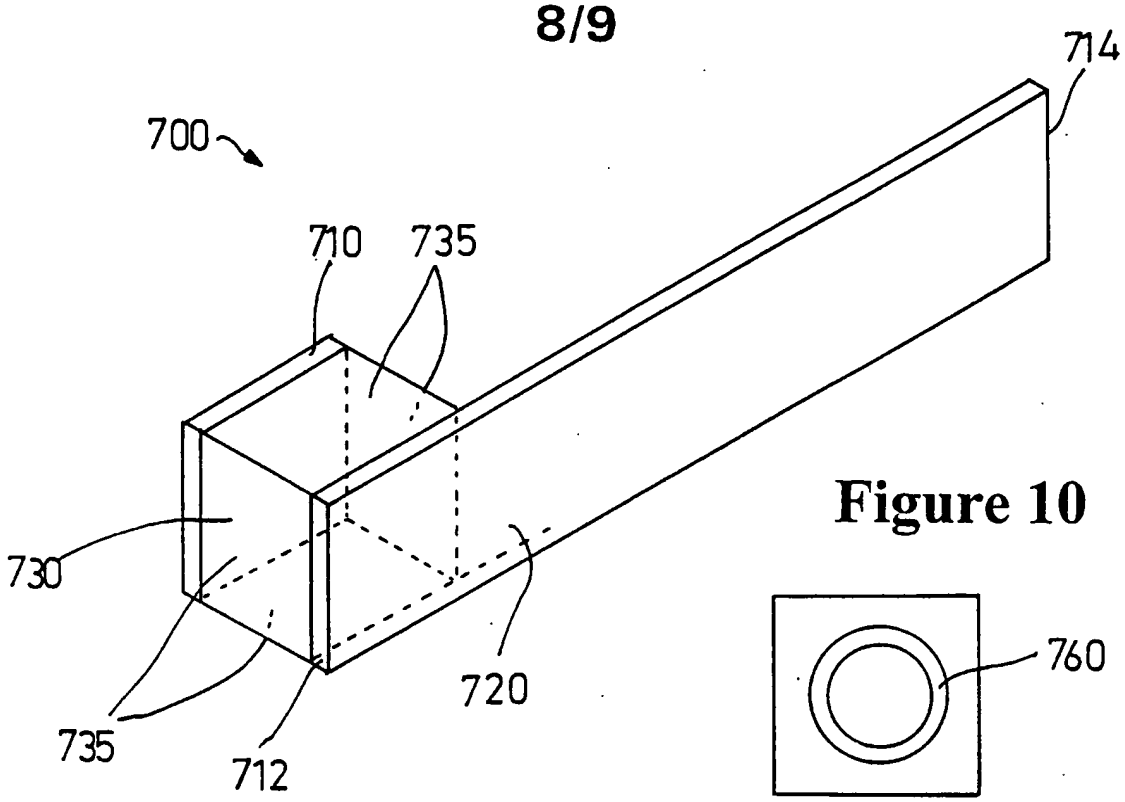

Figure 10

Figure 11(a)

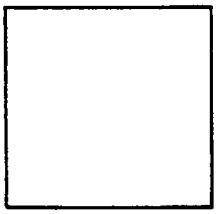

Figure 11(b)

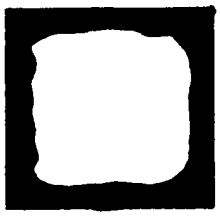

Figure 11(c)

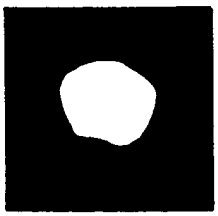

Figure 11(d)

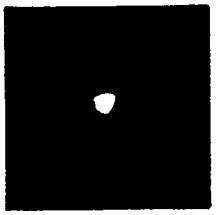

Figure 11(e)

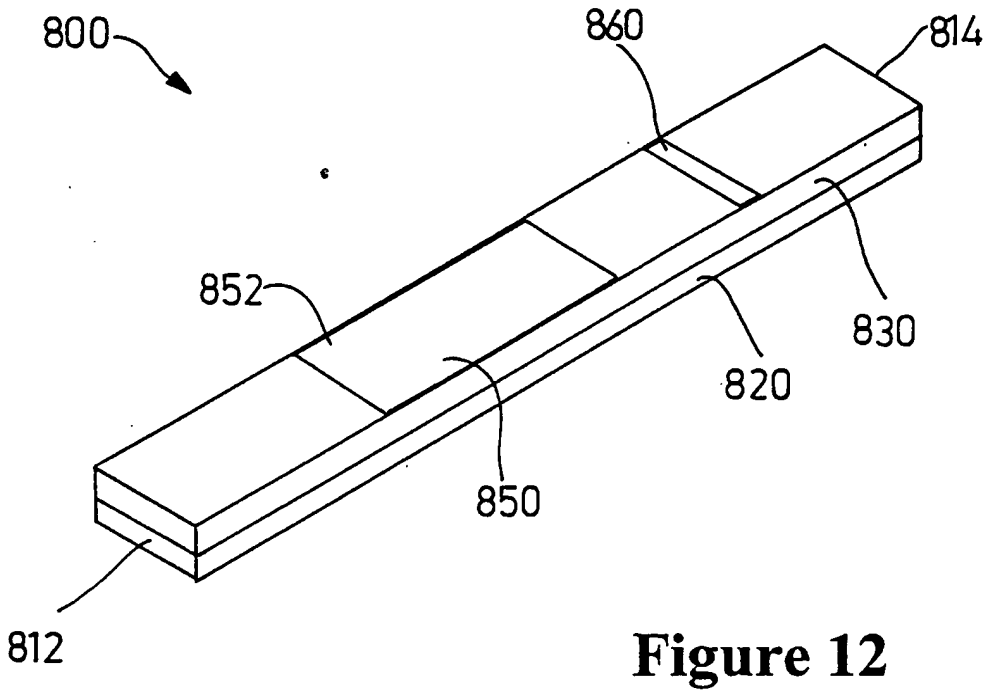

Figure 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00377

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/558
US CL :436/514
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,943,522 A (EISINGER et al) 24 July 1990, see entire document.	1-28
A	US 5,260,194 A (OLSON) 9 November 1993, see entire document.	1-28
A	US 5,308,775 A (DONOVAN et al) 3 May 1994, see entire document.	1-28
A, P	US 5,658,801 A (POISSANT et al) 19 August 1997, see entire document.	1-28
A	US 5,468,648 A (CHANDLER) 21 November 1995, see entire document.	1-28

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
11 FEBRUARY 1998

Date of mailing of the international search report
16 MAR 1998

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00377

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	US 5,559,041 A (KANG et al) 24 September 1996, see entire document.	1-28
A	US 5,354,692 A (YANG et al) 11 October 1994, see entire document.	1-28
A	US 5,384,264 A (CHEN et al) 24 January 1995, see entire document.	1-28
A	US 5,424,220 A (GOERLACH-GRAW et al) 13 June 1995, see entire document.	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL97/00377

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/56, 57, 58; 435/5, 7.2, 7.21, 7.32, 7.34, 7.36, 7.92, 287.1, 287.2, 287.7, 287.9, 805, 810, 962, 970, 974; 436/65, 164, 169, 500, 510, 514, 518, 524, 525, 526, 531, 533, 534, 805, 810, 814, 815, 816, 818, 901

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS
search terms: test strip, control zone, detection zone, migration, antibody, antigen, virus, streptococcus, hcg, human chorionic gonadotropin, pregnancy